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THE RESPONSES OF HAIR CELLS IN THE BASAL TURN OF THE GUINEA-PIG COCHLEA TO TONES

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SUMMARY

1. Intracellular recordings were made from inner and outer hair cells in the basal turn of the guinea-pig cochlea. The resting membrane potentials of the inner hair cells are more positive than −50 mV while those of outer hair cells are usually more negative than −70 mV.

2. At low frequencies the receptor potentials of inner hair cells are predominantly depolarizing while those from outer hair cells are hyperpolarizing at low and moderate sound pressure (e.g. < 90 dB re 2×10^{-5} Pa at 600 Hz). The potentials then become predominantly depolarizing at high sound pressure.

3. The asymmetry of the inner and outer hair cell receptor potentials are manifested instantaneously except at high stimulus levels when the depolarizing responses of outer hair cells take several cycles to develop.

4. At the offset of intense tones outer hair cell membrane potentials remain depolarized by 1–2 mV above their resting value and return to normal over a period depending on the level and duration of the tone.

5. In response to tones above about 2 kHz and at levels below about 90 dB the wave forms of outer hair cell receptor potentials are virtually symmetrical without measurable d.c. components. In response to tones close to their best frequencies (16–21 kHz), inner hair cells in the basal turn generate large depolarizing (d.c.) receptor potentials while outer hair cells from this region of the cochlea do not generate significant voltage responses.

6. Frequency tuning curves were derived for inner and outer hair cells from the amplitude–intensity relationships of their d.c. and phasic (a.c.) receptor potentials respectively. When the latter were compensated for the low-pass characteristics of the recording system and the hair cell time constant, the frequency selectivity of inner and outer hair cells are similar.

7. The response properties of inner and outer hair cells in the basal turn of the guinea-pig cochlea are discussed in relation to their proposed roles in mechano-electric transduction.

INTRODUCTION

There are two classes of hair cells in the organ of Corti of the mammalian cochlea, a single row of inner hair cells and generally three rows of outer hair cells. The two

classes of hair cells are morphologically distinct and are further distinguished from each other by their patterns of innervation; inner hair cells form synapses with over 95% of afferent fibres in the auditory nerve while outer hair cells are the targets of the axo-somatic terminals of the olivo-cochlear bundle (Morrison, Schindler & Wersall, 1974). Intracellular recordings have been made from hair cells in the guinea-pig cochlea but there is no detailed description of the characteristics of outer hair cells in the basal, high-frequency turn (Russell & Sellick, 1978, 1983; Tanaka, Asanuma & Yanagisawa, 1980; Goodman, Smith & Chamberlain, 1982; Dallos, Santos-Sacchi & Flock, 1982; Brown, Nuttall & Masta, 1983; Brown & Nuttall, 1984; Dallos, 1985). In this paper we describe the voltage responses of outer hair cells in the basal turn of the guinea-pig cochlea to low-frequency tones and to the high-frequency tones for which the basal turn of the cochlea is most sensitive. These responses and the tuning properties of the outer hair cells are compared with those of inner hair cells from the same region of the cochlea and assessed in the light of recent speculation about the roles of inner and outer hair cells in mechano-electric transduction in the cochlea.

METHODS

Animal preparation and electrical recording

Pigmented guinea-pigs weighing between 200 and 300 g were anaesthetized with sodium pentobarbitone (30 mg/kg), Operidine (1 mg/kg) and Droleptan (4 mg/kg) (Evans, 1979) and a tracheal cannula was inserted. Operidine was administered every 40 min and the body temperature of the guinea-pig was maintained at 38 °C with a heating blanket. The heart rate was monitored through a pair of skin electrodes placed either side of the thorax. The right cochlea was exposed through a lateral opening in the temporal bone and back-illuminated by a fibre optic light guide inserted through a hole made in the basal wall of the bulla. A small oval opening was gently scraped in the ventro-lateral wall of the basal turn of the cochlea with a chisel-shaped scalpel blade to reveal the scala tympani and basilar membrane. All experiments were performed in an electrically shielded sound-proofed room on a vibration-isolated table.

Micropipettes were pulled with a Brown–Flaming model P-77 puller from 1 mm o.d. fibre-filled glass tubing (Clark Electromedical) and filled with 3 M-KCl. Resistances of the pipettes ranged between 100 and 140 MΩ when measured in the perilymph of the scala tympani. The micropipettes were connected to a 20 dB gain, high-impedence, d.c.-coupled pre-amplifier with capacity compensation and facilities for current injection and bridge balancing. The Ag–AgCl indifferent electrode was inserted in the neck muscles. This could be either connected to ground or through a current–voltage converter to measure the current injected through the micropipette.

The compound action potential (c.a.p.) of the cochlear nerve was recorded with an electrode placed on the round window. These responses were amplified with a high-gain (60 dB), a.c.-coupled amplifier with a pass band of 20 Hz to 4 kHz. The amplified responses of the micropipette and round-window electrode were stored on an FM tape recorder (Racal Store 4DS).

Acoustic stimulation

Sound was delivered to the tympanic membrane by a calibrated, closed acoustic system. High-frequency tones were delivered through a Bruel & Kjaer 3134 1/2 in condenser microphone, and a Beyer DT48 dynamic ear-phone was used for delivering tones below 4 kHz. A calibrated 1/2 in probe microphone with 1 mm probe tube positioned 1 mm from the tympanic membrane was used for *in situ* calibration. The transfer ratio of the sound pressure at the tympanic membrane to voltage into the ear-phone was measured as a function of frequency at the beginning of each experiment. Using this transfer ratio, continuous tones and tone bursts were presented at a known sound level, expressed in this paper in dB sound pressure level (s.p.l.; dB re 2×10^{-5} Pa).

Tones were generated by a computer-controlled function generator (Hewlett–Packard 3235A).

Tone bursts were produced by gating the continuous tone at zero crossing with a trapezoidal envelope (rise time and duration 1 and 50 ms respectively).

Experimental procedures

The sensitivity of the cochlea was assessed by constructing an audiogram based on the visual detection threshold of the c.a.p. to pure tones over the frequency range 1–22 kHz. This was done before and after exposure of the basilar membrane, and following the penetration of each hair cell. In the experiments described here exposure of the basilar membrane did not result in a threshold loss of more than 10 dB. Thresholds sometimes suddenly increased following penetration of the basilar membrane, particularly in the region of the outer hair cells and, if the threshold loss was greater than 20 dB, the experiment was terminated.

The micropipettes were advanced towards the basilar membrane with a custom-built hydraulic microdrive (Evans, 1979). Contact with the basilar membrane was indicated by the increase in the voltage response of the recording system to a +0·15 nA pulse injected at the pre-amplifier input. The electrode was backed away a few micrometres, the capacitance was adjusted and the frequency response of the electrode was determined (from the rise time of the voltage response to current pulse). The electrical linearity of the recording system was assessed by injecting alternate positive and negative current steps in 0·15 nA increments up to 1·8 nA at the pre-amplified input and measuring the out-of-balance voltage response of the pre-amplifier. If the electrodes were strongly rectifying or excessively noisy they were rejected.

Frequency response of the recording system

We compared the frequency dependence of the voltage transfer function (ratio of the voltage at the pre-amplifier output to the voltage at the electrode tip) and the transfer impedance of our recording system (micro-eletrode and pre-amplifier) both in the test rig described by Baden-Kristensen & Weiss (1983) and in the perilymph of the scala tympani. In agreement with their findings and with a theoretical analysis (Weiss, Peake & Sohmer, 1971), the recording system behaved approximately as a low-pass filter whose magnitude was constant at low frequencies and decreased at a rate of about 12 dB per octave for frequencies above the corner frequency (the frequency at which the magnitude is 3 dB below its value at low frequencies). To measure the frequency response of the recording system in the scala tympani we first advanced the electrode until it was just above the basilar membrane, measured the electrode resistance, injected a square wave of current at the pre-amplifier input and adjusted the capacitance compensation. Sinusoidal current at frequencies between 100 Hz and 24 kHz and 0·1 nA in amplitude was then injected at the input of the pre-amplifier to measure the transfer impedance. These measurements had corner frequencies in the range 3·5 to 4·5 kHz, and for each electrode there was close correspondence between this and the cut-off frequency estimated from the rise time (time taken for the wave form to reach 80–90 % of its final value) of a current step injected at the pre-amplifier inputs (Baden-Kristensen & Weiss, 1983).

Signal processing

The voltage responses to tones were sampled at 200 μs intervals with a Plessey PDP11-03 microcomputer system and averaged (n = 6–8). Continuous tone responses were sampled with a pair of lock-in amplifiers (Brookdeal 9504SC) and the phase and modulus of the fundamental and second harmonic were measured with an Omniphase module.

Identification of hair cell responses

We have recorded the resting membrane potentials and their responses to low- and high-frequency tones from over 100 putative inner and outer hair cells with micropipettes filled with either 6 % Procion or 3 % Lucifer Yellow dye (Stewart, 1978). In most experiments it was possible to fill only one cell. The cochleas were prepared for histology according to the method described by Russell & Sellick (1978) and the dye-marked cells were observed in 10 μm sections of epoxy-embedded cochleas with a fluorescence microscope (see Fig. 1). Our success in confirming the identity of putative inner and outer hair cells was 100 % although the retrieval rate was only 21 % (twenty-eight inner) and 15 % (three outer). The remaining cells were lost either in preparation or were not filled with dye.

It is potentially possible to confuse the responses of outer hair cells and those of supporting cells

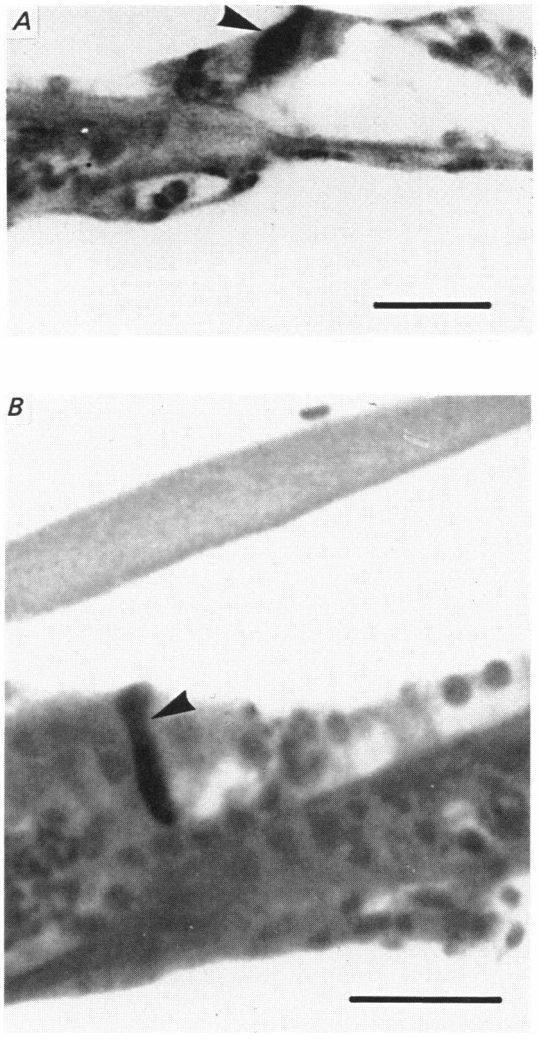

Fig. 1. *A*, transverse section through the basal turn of the cochlea showing a Procion-Yellow-filled inner hair cell (arrow). *B*, tangential section through the basal turn of the cochlea at the level of the inner row of outer hair cells showing a Lucifer-Yellow-filled outer hair cell (arrow). Scale bars 20 μm.

because the wave forms of the outer hair cell receptor potentials and the cochlear microphonic (c.m.) are similar. However, in response to tones of a few hundred hertz, or less, the intracellular responses of the outer hair cells are 2–3 times greater than the c.m., while in response to tones of 10 kHz or greater, the c.m. recorded extracellularly in the organ of Corti and intracellularly in the supporting cells is 2–20 times greater than the outer hair cell receptor potentials. This is because the input resistance of outer hair cells (20–40 MΩ) is much larger than that of the supporting cells (Santos-Sacchi & Dallos, 1983; Santos-Sacchi, 1984; A. R. Cody & I. J. Russell, unpublished observations). Furthermore the c.m. recorded from supporting cells is unchanged by current injection while the receptor potentials of outer hair cells are increased by hyperpolarizing currents and decreased by depolarizing current injection.

RESULTS

The resting membrane potentials

Inner and outer hair cells are believed to subserve the same task, which is mechano-electric transduction of basilar membrane motion; however, their passive electrical properties and responses to tones are different. The differences are first apparent from measurements of their steady-state resting membrane potentials which, in inner hair cells, are usually more depolarized than −45 mV (mean ± S.D., $-37 \pm 6{\cdot}8$ mV, $n = 21$) while those in outer hair cells are usually more hyperpolarized than −70 mV ($-83 \pm 12{\cdot}8$ mV, $n = 20$). We have not observed the systematic differences in the initial and steady-state values of the resting potentials described by Dallos (1985) for hair cells in the apical turns of the cochlea. In this paper we have restricted the sample of cells to those whose resting membrane potentials remained stable during the recording period (30 s to 30 min).

The responses of hair cells to low-frequency tones

The voltage responses of hair cells to tones are the phasic a.c. and tonic d.c. receptor potentials (Russell & Sellick, 1978) which are the intracellular equivalents of the c.m. (Wever & Bray, 1930) and summating potential respectively (Davis, Fernandez & McAuliffe, 1950). In order to describe the phasic responses of hair cells to tones, the frequencies of the tones must be in the pass band of the recording system and cell membrane filter (Russell & Sellick, 1978). For this reason we have limited our description of the wave forms of hair cell receptor potentials to tones below 700 Hz (usually 600 Hz) because the upper frequency limit of the recording system was about 3·5 kHz and that of the hair cells was estimated to be at least 700 Hz (based on measurements of the rise times of the voltage responses of the hair cells to 0·1 nA current pulses). The higher harmonic components, but not the fundamental frequency, of responses to 600 Hz tones are attenuated by the low-pass filter characteristics of the cell membrane and recording system.

Fig. 2 shows the voltage responses of an inner hair cell and an adjacent outer hair cell in the basal turn of the cochlea to tones at 600 Hz; a frequency which is well below the most sensitive or best frequency (about 19 kHz) of the cells illustrated. The receptor potentials of inner hair cells (Fig. 2*A*) may reach peak-to-peak amplitudes of about 30 mV. They are larger than those of outer hair cells (Fig. 2*C*) by a factor of between 2 and 3, and they are asymmetrical, being larger and more peaked in the positive direction. The negative phase of the receptor potential is more rounded and tends to saturate at lower stimulus levels (Fig. 2*B*). At low intensities the receptor potentials of outer hair cells (Fig. 2*C*) are almost symmetrical, but with increasing sound pressure they become asymmetrical in the opposite direction to the inner hair cells with the hyperpolarizing phase being larger than the depolarizing phase.

The stimulus–response relationships of hair cells

The peak voltage responses and peak sound pressure for inner and outer hair cells are plotted adjacent to their intracellular records in Fig. 2*C* and *D*. On the basis of earlier experiments (Sellick & Russell, 1980; Russell & Sellick, 1983), in which it was established that inner hair cells are excited during the rarifaction of a tone, we have

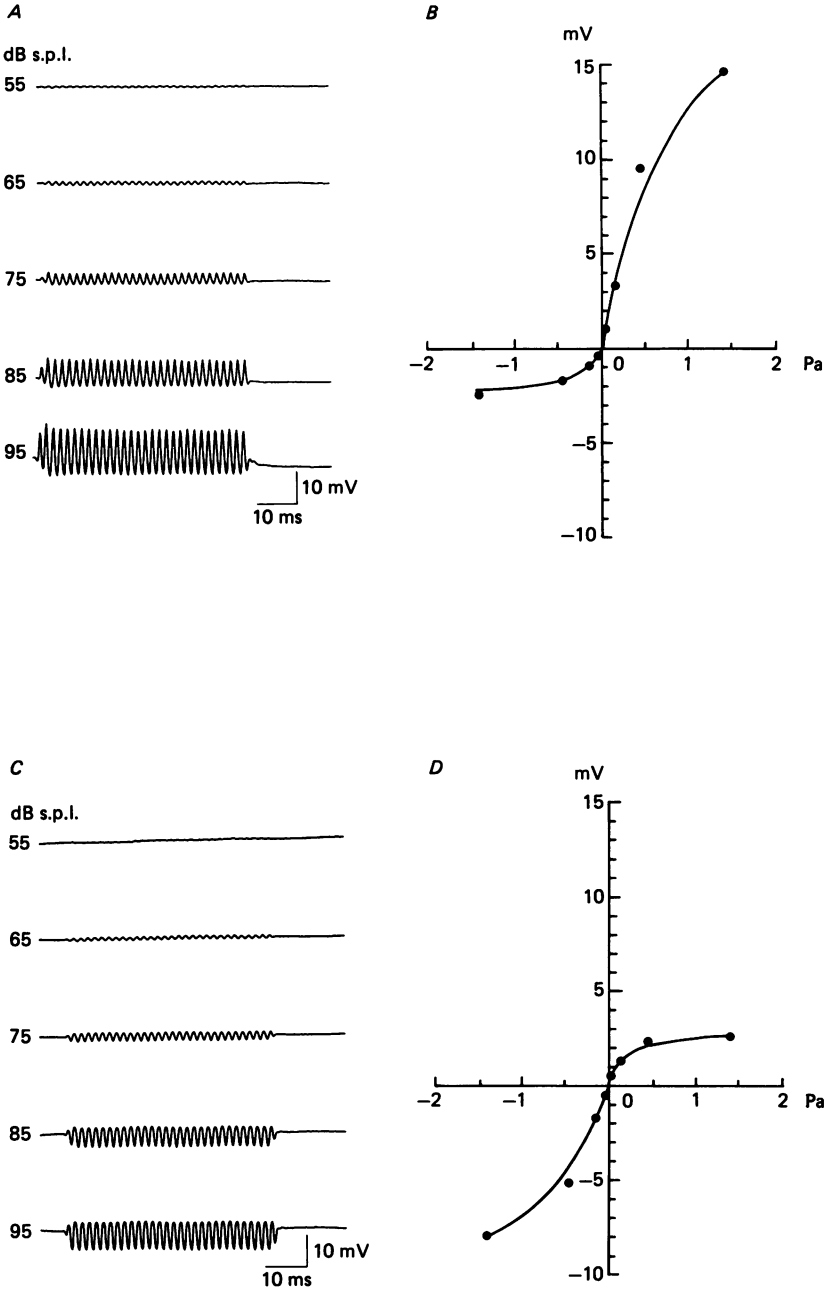

Fig. 2. The averaged voltage responses ($n = 4$) of an inner hair cell (A) and an adjacent outer hair cell (C) from the basal turn of the same cochlea, to 600 Hz tones at the sound pressures indicated. B and D show the relationship between the peak voltage responses (mV) and peak sound pressure (Pa) for rarifaction (depolarization) and compression (hyperpolarization) for the inner and outer hair cells whose averaged responses are shown in A and C respectively. The curves through the points are composed of two rectangular hyperbolae.

adopted the convention that rarifaction is positive and associated with the depolarizing phase of the receptor potential. The smooth curves which have been drawn through the points are composed of two rectangular hyperbolae whose constants were determined from the linear representation of the data (Eadie, 1942; Russell & Sellick, 1983). Rectangular hyperbolae have been fitted to the input–output relationships of a variety of sense organs including those of the acoustico-lateralis system (Boston, 1980; Crawford & Fettiplace, 1981; Russell & Sellick, 1983). We did

TABLE 1. The properties of receptor potentials (in response to 600 Hz tones) recorded from inner and outer hair cells in the basal turn of the cochlea derived from the fit (by linear regression, Eadie, 1942) of rectangular hyperbolae to their amplitude–intensity relationships similar to those in Fig. 2

	Inner hair cell	Outer hair cell
Number of cells	6	6
Depolarization–hyperpolarization	5·7 ± 2·2	0·6 ± 0·3
Peak-to-peak amplitude at 90 dB s.p.l. (mV)	20·3 ± 6·1	9·7 ± 1·6
Sound pressure for 50% maximum positive response (Pa)	0·5 ± 0·1	0·23 ± 0·1
Sound pressure for 50% maximum negative response (Pa)	0·4 ± 0·1	0·8 ± 0·2
Correlation coefficient	> 0·96	> 0·96

not compare our data with the three-state model of Corey & Hudspeth (1983) which successfully describes the relationship between stereocilia displacement and receptor current in hair cells of the frog sacculus. This is because we have no direct measure of stereocilia displacement in cochlear hair cells or any idea how this might change during intense tones (Cody & Russell, 1985).

Pairs of rectangular hyperbolae were fitted to the stimulus–response relationships of twelve other hair cells (six inner and six outer) with high correlation coefficients (> 0·96). The constants of these rectangular hyperbolae are shown in Table 1. For responses to 600 Hz tones at 90 dB or less, the depolarizing phase of the receptor potential recorded from outer hair cells saturates at lower sound pressures than its counterpart in inner hair cells. The converse is true for the hyperpolarizing phase.

For very intense stimuli (e.g. 110 dB s.p.l. at 600 Hz) the asymmetry of the outer hair cell responses reverse and the depolarizing phase dominates (Fig. 3*A*). This reversal in polarity is a consequence of differences in the characteristics of the stimulus–response relationships of the positive and negative phases of the outer hair cell receptor potentials. When plotted on the same axes (Fig. 3*B*), with the sign of the negative responses reversed, it can be seen that the relationship of the positive phase is shifted downwards with respect to that of the negative phase. The positive phase of the receptor potential for this cell ultimately saturates at a higher sound pressure and amplitude than the negative phase, thereby yielding receptor potentials which are predominantly hyperpolarizing below 85 dB s.p.l. and depolarizing above this. In some cells the slopes of the stimulus–response relationships of the positive and negative phases of the receptor potential become reduced between 70 and 80 dB

s.p.l. However, above about 80 dB s.p.l., the slope of the positive phase of the receptor potential increases before saturating at higher levels. In other cells the stimulus–response relationships appear to grow smoothly throughout their dynamic range without an inflexion in the curve. It was usually possible to make recordings from only one or two inner hair cells in a preparation. Thus we are unable to say if the variability we have observed is a consequence of differences between cells, animals or in cochlear surgery resulting in small changes (< 10 dB s.p.l.) in c.a.p. sensitivity.

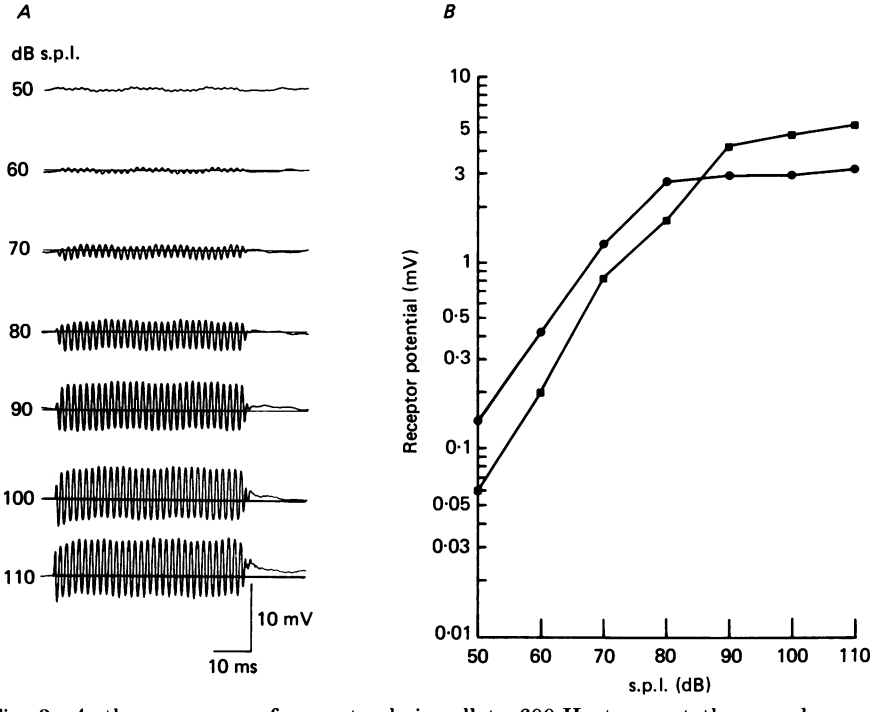

Fig. 3. *A*, the responses of an outer hair cell to 600 Hz tones at the sound pressures indicated to show the reversal in polarity of the receptor potentials during intense tones. The horizontal line through the records indicates the resting membrane potential (−85 mV). *B*, the relationship between the amplitude of the peak negative (●) and peak positive (■) receptor potentials (with respect to membrane potential) and sound pressure for the receptor potentials shown in *A*.

Unlike their responses to tones at lower sound pressures in which the hyperpolarizing asymmetry is instantly present, the depolarizing asymmetry may take several cycles to become established (Fig. 3*A* and 4). The membrane potentials of inner hair cells fall instantly to their resting levels following a tone burst and so do those of outer hair cells for low or moderately intense tones. This is not so for outer hair cells when exposed to tones intense enough to cause them to generate depolarizing receptor potentials. At the offset of the tone the outer hair cell membrane potentials remain depolarized above their resting levels (Fig. 3*A*) for a period which appears to be related to the duration and intensity of the tone. This relationship was not explored in these experiments, but for a 50 ms 600 Hz tone at 114 dB s.p.l., the post-stimulus

depolarization of the outer hair cell membrane potential is about 1 mV and takes some 50 ms to recover.

Responses of hair cells to high-frequency tones

The dominant voltage response of inner hair cells in the basal turn to frequencies in the tip region of their tuning curves is the depolarizing d.c. receptor potential. This is a residual response which is thought to be the product of asymmetrical inner hair cell transducer conductance and the low-pass filter characteristics of the cell

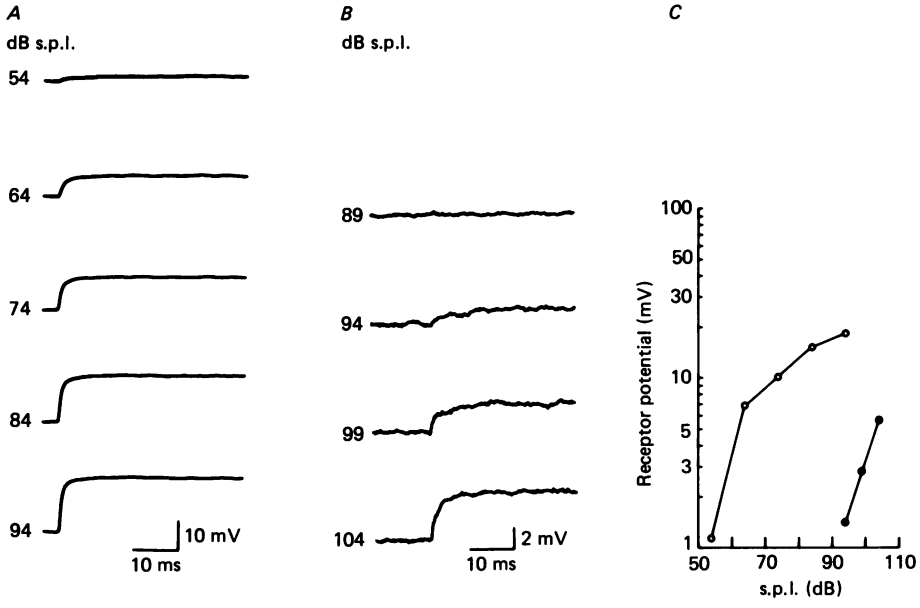

Fig. 4. *A*, inner hair cell d.c. receptor potentials in response to 16 kHz tones at the sound intensities shown. Resting potential, −40 mV; best frequency, 18 kHz. *B*, d.c. receptor potentials from an outer hair cell located close to the inner hair cell in *A* in response to 16 kHz tones. Resting potential, −85 mV. *C*, stimulus–response functions of the inner (○) and outer (●) hair cell d.c. receptor potentials shown in *A* and *B*.

membrane (Russell & Sellick 1978). Examples of d.c. receptor potentials to tones at 16 kHz and their stimulus–response relationships are shown in Fig. 4*A* and *C* for an inner hair cell whose best frequency is 18 kHz. The potentials are large, sometimes exceeding 20 mV in response to intense tones at frequencies about one half an octave below their best frequency (Cody & Russell, 1985). At intensities corresponding to the visual detection threshold of the c.a.p. recorded at the round window to pure tones, the inner hair cell d.c. receptor potential is about 2 mV in amplitude. The threshold for excitation of auditory afferent fibres is about 10 dB below the c.a.p. threshold (Dallos, Harris, Ozdamar & Ryan, 1978; Ozdamar & Dallos, 1976). Thus, at neural threshold, the d.c. receptor potential would be about 0·8 mV in amplitude.

The reversed asymmetry of the outer hair cell receptor potentials is conspicuous only at frequencies below about 1 kHz. In response to tones above about 2 kHz and at sound pressures below about 90 dB, the receptor potentials are virtually

symmetrical (Fig. 5) and the d.c. components are immeasurably small. These frequency- and level-dependent responses of outer hair cells to acoustic stimulation were recorded in cochleas which showed no measurable change in sensitivity during and following intracellular recording. Furthermore, in five comparative studies, the frequency selectivity of the outer hair cells did not differ significantly from those of adjacent inner hair cells (see below). From these experiments we conclude that the generation of receptor potentials without a d.c. component to tones of moderate intensity at frequencies above about 2 kHz is a natural property of outer hair cells in the basal turn of the guinea-pig cochlea and not a consequence of experimental perturbation. Thus it appears that the transfer characteristics of outer hair cells in the basal turn, like those in the apical turn (Dallos *et al.* 1982; Dallos, 1985) are frequency dependent. However, they differ in that, at low or moderate intensities, only outer hair cells in the apical turn of the cochlea generate asymmetrical receptor potentials with positive d.c.

Outer hair cells do generate d.c. receptor potentials in response to tones close to their best frequencies, but only when the sound pressure exceeds about 90 dB. Examples are shown in Fig. 4*B* where they may be compared with those of the inner hair cell recorded close by in the same cochlea. The d.c. receptor potentials of outer hair cells are invariably depolarizing at these frequencies and are measurable only at stimulus levels where the inner hair cell receptor potentials are obviously saturating. In addition the outer hair cell d.c. receptor potentials are much smaller than those of the inner hair cells and rarely exceed 5 mV. The stimulus–response relationships of the inner and outer hair cell d.c. receptor potentials for tones at 16 kHz, which are plotted in Fig. 4*C*, have initial slopes of about 2. Thus in some, but not all, hair cells from which we have made recordings, the initial growth rate of the d.c. potentials contrasts with that of the peak-to-peak amplitude of the receptor potentials, evoked by low-frequency tones, which grow in proportion to sound pressure level or stimulus amplitude (Fig. 3).

The rise times of the outer hair cell d.c. receptor potentials are slower and level dependent, unlike those of inner hair cells whose rise times are coincident with the tone burst envelope (Fig. 4*A*). We have not attempted to study the dependence of the rise times of the outer hair cell d.c. responses on stimulus level but for the typical examples shown in Fig. 4*B*, the rise times vary from about 6 ms at 94 dB s.p.l. to about 2·5 ms at 114 dB s.p.l. The development of a slow net depolarization in the outer hair cell membrane potential with increasing stimulus level can also be seen in Fig. 5 where the responses of an outer hair cell to 2 kHz tones (Fig. 5*A*) are compared with d.c. components (shown in Fig. 5*B*).

The phasic voltage responses of outer hair cells to tones close to their best frequency are, like those of inner hair cells, drastically attenuated by the cell membrane filter. Outer hair cells in the basal turn generate receptor potentials of 10–30 μV in amplitude at the threshold for neural excitation for tones at frequencies between 16 and 18 kHz. These measurements take into account the low-pass filter characteristics of the recording system. So, with the exception of their responses to very intense stimuli, outer hair cells in the basal turn of the cochlea do not generate significant voltage responses to tones at the frequencies to which this region is most sensitive.

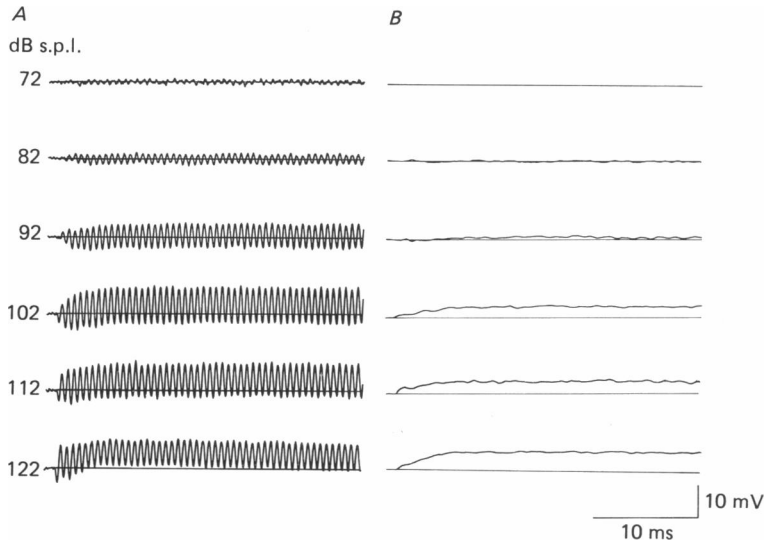

Fig. 5. *A*, outer hair-cell receptor potentials in response to 2 kHz tones. The horizontal lines through each trace indicate the resting membrane potential (−80 mV). *B*, the d.c. components of the receptor potentials shown in *A*. These were obtained by measuring the half-peak-to-peak amplitudes of the receptor potentials shown in *A* (the error in this approximation of the wave-form integral was less than 5%).

The frequency selectivity of inner and outer hair cells

We have compared the stimulus–response relationships of the d.c. receptor potentials of inner hair cells and the a.c. receptor potentials of outer hair cells recorded close to each other in the basal turn of the same cochlear and derived iso-response tuning curves from them. The aim of these experiments was to compare the frequency tuning of inner and outer hair cells under similar recording conditions and cochlear sensitivity. For this reason the frequency selectivity of the inner hair cells was first determined as rapidly as possible from measurements of their d.c. receptor potentials and the micropipette was then repositioned to record the a.c. receptor potentials from the outer hair cells. This experimental sequence was chosen because there is less chance of damage to the organ of Corti when recording from inner hair cells.

The d.c. receptor potentials are residual voltage responses which are not attenuated by the low-pass filters of the hair cell membrane or the recording system. The a.c. receptor potentials recorded from the outer hair cells are attenuated by both filters. Therefore, in order to compare the intensity relationships and the derived iso-response tuning curves of the inner hair cell d.c. responses with the a.c. responses of the outer hair cells, the outer hair cell a.c. receptor potentials were compensated for the attenuation due to the cell membrane filter (6 dB per octave above 1·2 kHz, which was the estimated corner frequency of the cell, based on the rise time of its voltage response to a 0·1 nA current step) and that due to the recording system (12 dB per octave above 3·5 kHz). Errors in estimating the cut-off frequency of the membrane filter and recording system will have little influence on the apparent shape of the tip

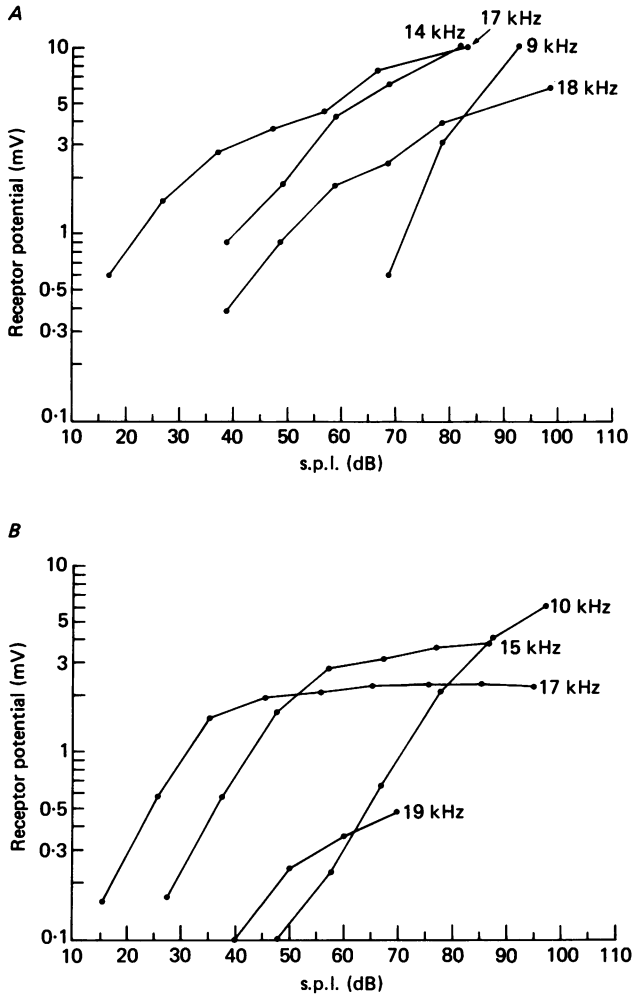

Fig. 6. Stimulus–response relationships for the d.c. receptor potentials of an inner hair cell (*A*) and the a.c. receptor potentials of an outer hair cell (*B*) at the frequencies indicated by each curve. The intracellular recordings from the two hair cells were made in the same cochlea and within a few minutes of each other. The a.c. responses recorded from the outer hair cell were compensated for the attenuation due to the cell time constant (6 dB per octave above 1·2 kHz) and that due to the recording system (12 dB per octave above 3·5 kHz).

of the tuning curve where the high- and low-frequency slopes are very steep compared with those of the low-pass filters (see Table 2). However, errors will influence the apparent values of the s.p.l. at which the responses are generated. In the example illustrated in Figs. 5*B* and 6 the correction applied to the a.c. receptor potentials of the outer hair cell at its characteristic frequency is about 23 dB for the membrane filter and 26 dB for the recording system. An under- or over-estimation of the recording system time constant by an octave will cause the outer hair cell iso-response tuning curve to appear 12 dB more or less sensitive at the tip, respectively, and this

error will be compounded by errors in estimating the cut-off frequency of the membrane filter.

The stimulus–response relationships for the d.c. receptor potentials of an inner hair cell and the frequency-compensated a.c. receptor potentials of an outer hair cell recorded close to each other in the same cochlea are shown in Fig. 6*A* and *B*. It is apparent that as the tone frequencies approach the best frequency of the cell and exceed it, the responses of the hair cells saturate at progressively lower amplitudes. This compressive frequency-dependent non-linear behaviour is characteristic of the mechanical properties of the basilar membrane (Sellick, Patuzzi & Johnstone, 1982; Khanna & Leonard, 1982; Robles, Ruggero & Rich, 1985) and the hair cell receptor potentials (Russell & Sellick, 1978; Goodman *et al.* 1982; Brown *et al.* 1983; Dallos, 1985).

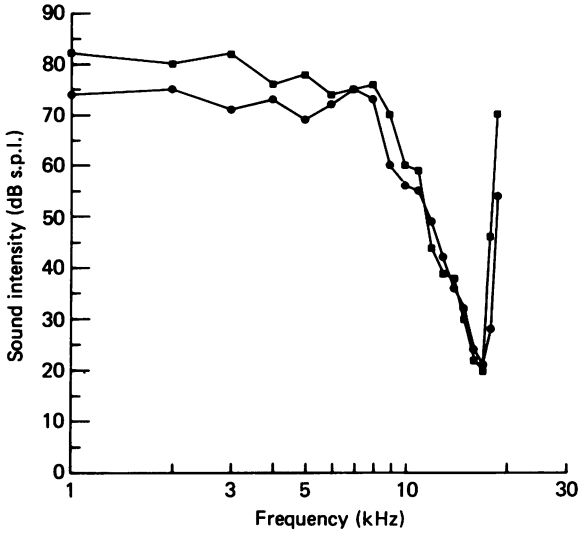

Fig. 7. Iso-response tuning curves of the inner (■, response criterion 0·8 mV) and outer hair cell (●, response criterion 0·3 mV) whose amplitude–intensity functions are shown in Fig. 6. The a.c. responses recorded from the outer hair cell were compensated for the attenuation due to the cell time constant (6 dB per octave above 1·2 kHz) and that due to the recording system (12 dB per octave above 3·5 kHz).

The iso-response tuning curves were derived from the intensity functions by selecting as a criterion the amplitude of receptor potentials generated by the inner and outer hair cells at the visual detection threshold of the c.a.p. at their best frequency. For the inner and outer hair cell illustrated in Fig. 7 this was 0·8 mV for the d.c. receptor potentials and 0·3 mV for the frequency-compensated a.c. receptor potentials respectively. The frequency response curves of the inner and outer hair cells were found to be very similar in four other preparations where it was possible to record from an inner hair cell and an adjacent outer hair cell without change in cochlear sensitivity. The filter characteristics of the inner and outer hair cells presented in Table 2 are comparable to those described for inner hair cells by Russell & Sellick (1978) and it is concluded that the tuning of the mechanical inputs to the two types of hair cells is very similar.

TABLE 2. Filter characteristics of inner and outer hair cells in the basal turn of the cochlea. The outer hair cell parameters are based on tuning curves for the a.c. component, corrected for the attenuation produced by the membrane time constant and recording system, whereas the inner hair cell parameters are derived from tuning curves of the d.c. component

	Inner hair cell	Outer hair cell
Number of cells	5	5
Range of best frequencies (kHz)	16–21	16–21
$Q_{10,dB}$ (centre frequency/bandwidth 10 dB above tip)	6·9±1·4	6·9±1·1
High-frequency slope (dB per octave)	503±124	466±119
Low-frequency slope (dB per octave)	155±50	154±30
Tip to shoulder (dB)	58±6	53±8

DISCUSSION

From our results we conclude that the frequency-tuning characteristics of inner and outer hair cells in the basal turn of the guinea-pig cochlea are remarkably similar to each other but in other respects their response characteristics are quite different and may reflect different roles for inner and outer hair cells in mechano-electric transduction in the cochlea. The resting membrane potentials of outer hair cells are more negative than those of the inner hair cells and the polarity of the outer hair cell voltage responses to low-frequency tones are level dependent while the receptor potentials of inner hair cells are always depolarizing. The most remarkable difference is in their response to high-frequency tones. Inner hair cells generate d.c. receptor potentials while the voltage responses of outer hair cells to high-frequency tones are insignificant.

Frequency tuning of the outer hair cells

The iso-response tuning curves we have derived from the stimulus–response characteristics of inner and outer hair cells in the basal turn of the cochlea are remarkably similar to each other, to the mechanical tuning of the basilar membrane (Sellick *et al.* 1982) and to neural tuning curves (Evans, 1972). This result is not surprising in the context of current speculation that outer hair cells are primarily responsible for frequency selectivity and sensitivity in the cochlea (see Dallos, 1981, and Pickles, 1985, for reviews). However, the sharp frequency selectivity of outer hair cells is difficult to equate with the broad frequency tuning of the c.m. (Dallos, 1973). This apparent ambiguity has recently been resolved by Patuzzi (1986) who has shown that the broad tuning properties can be explained by taking into account characteristics which are known to be present in cochlear transduction such as spatial summation, phase cancellation, progressive saturation of the receptor currents through the outer hair cells and frequency-dependent growth of the amplitude of vibration of the partition.

The resting membrane potential

The resting membrane potentials of inner hair cells are more depolarized than those of the outer hair cell, a point which has been noted before from intracellular recordings

in the basal and apical turns (Dallos *et al.* 1982; Russell & Sellick, 1983; Dallos, 1985). The differences in the membrane potentials of the inner and outer hair cells may have significance for the transmission of sensory signals across their afferent synapses. If the transfer characteristics of the afferent synapses of inner and outer hair cells are similar, then their operating points will be different. The spontaneous activity of the type I afferent fibres (Kiang, Watanabe, Thomas & Clark, 1965; Evans, 1972) is an indication that the inner hair cell afferent synapses operate above their threshold for transmitter release. However, it is possible that, in the absence of sound, the afferent synapses of outer hair cells operate in a region below the threshold for transmitter release. At present there is virtually no evidence on this point. In one report of an intracellular recording from a morphologically identified type II neurone in the spiral ganglion of a guinea-pig cochlea, the neurone was found to be silent and not excited by acoustic stimulation (Robertson, 1984).

Outer hair-cell receptor potentials

A common feature of hair-cell receptor potentials is their depolarizing asymmetry (Flock & Russell, 1976; Hudspeth & Corey, 1977; Sellick & Russell, 1980; Crawford & Fettiplace, 1981; Russell, Richardson & Cody, 1986), an apparently inherent property of the transduction mechanism (Hudspeth & Corey, 1977) and not, for example, a consequence of an imposed mechanical bias. The asymmetry of the inner hair-cell receptor potential results in the production of the positive d.c. receptor potential which is their principal voltage response to high-frequency tones (Russell & Sellick, 1978; 1983).

The receptor potentials of outer hair cells recorded in the basal turn of the guinea-pig cochlea to tones well below their best frequencies are exceptional because, over a range extending to about 40–50 dB above their visual detection threshold (e.g. from about 50 to 90 dB s.p.l. at 600 Hz), they develop a negative asymmetry. Outer hair cells in the apical turns also have a reversed symmetry at frequencies below their best frequency, but over a more limited dynamic range, e.g. from about 45 to 55 dB s.p.l. at 300 Hz for a cell whose best frequency is 800 Hz (Dallos, 1985). At frequencies around 2 kHz, and above, and for intensities below about 90 dB s.p.l., outer hair cells in the basal turn do not appear to generate d.c. components. This implies that the wave forms of the outer hair-cell receptor potentials are symmetrical at these frequencies and intensities and this is borne out by the responses of the outer hair cells to tones at 2 kHz where the a.c. components can be directly observed (Fig. 5). Outer hair cells in the apical turn generate depolarizing potentials to tones at their best frequencies (Dallos *et al.* 1982; Dallos, 1985), but in the basal turn outer hair cells generate depolarizing response only to intense tones. In the basal turn, at least, the appearance of large (*ca.* 5 mV) depolarizing receptor potentials in the outer hair cells generate depolarizing responses only to intense tones. In the basal turn, at least, of the adjacent inner hair cells (Cody & Russell, 1985).

The positive asymmetry in the receptor potentials in basal turn outer hair cells during intense tones is not manifested instantaneously but takes a period of time which decreases with increasing intensity. This may indicate that the process maintaining the hyperpolarizing asymmetry breaks down during intense tones at a rate which is level dependent. At the offset of the intense tones, the outer hair-cell membrane potentials do not return instantly to their pre-stimulus levels, but to levels

more positive than this. They repolarize over a period of time which appears to be governed by the intensity and duration of the stimulus (Cody & Russell, 1985). The basis for this depolarization is not known, but one possible cause might be the accumulation of potassium in the spaces of Nuel surrounding the outer hair cells. This is because the receptor current in cochlear hair cells is carried by potassium ions (Russell, 1983) which flow through the transducer channels as a result of the potential gradient created across the sensory membrane of the hair cells by their membrane potentials and the endocochlear potential (Davis, 1958). The gradient for the efflux of potassium from the hair cells is provided by the relatively low extracellular concentrations of this ion in the perilymph (Johnstone & Sellick, 1972). If the rate-limiting step in this process is the removal of potassium ions from the spaces of Nuel, the resulting increase in concentration of extracellular potassium, as a consequence of intense acoustic stimulation, will depolarize the outer hair cells. It is of interest to test this hypothesis in the light of recent mechanical measurements by Zenner, Zimmermann & Schmitt (1985) on isolated outer hair cells which show that they contract in length when exposed to high potassium concentrations. If the depolarization we have observed is due to the accumulation of extracellular potassium, then predictably the depolarization should be associated with a change in the mechanical properties of the cochlea and a change in its sensitivity. In this respect we have observed that the recovery of inner hair-cell receptor potentials parallels the repolarization of the outer hair-cell membrane potentials following acoustic overstimulation (Cody & Russell, 1985).

To what extent is the reversed symmetry of the outer hair-cell receptor potentials a product of the intrinsic non-linear properties of the hair cells or of their mechanical input? In the absence of any direct measurements of the mechanical input to cochlear hair cells *in vivo* and the accumulating but indirect evidence that transduction in the cochlea is a bidirectional process involving electro-mechanical feed-back (Kemp, 1979; Mountain, 1980; Sellick *et al.* 1982; Khanna & Leonard, 1982; Weiss. 1982; Davis, 1983; Neely & Kim, 1983; Brownell, Bader, Bertrand & Ribaupierre, 1985), this question is rather difficult to deal with. First, we might consider the possibility that the reversed symmetry is due to a transduction process in the outer hair cells which differs from that in other hair cells. If this was so then the symmetry of the transduction mechanism in outer hair cells would have to be frequency and intensity dependent. Studies of the responses of inner and outer hair cells to direct displacement of their stereocilia in mouse cochleas maintained *in vitro* show that this is not the case. In this preparation the receptor potentials of the outer hair cells are similar to those of the inner hair cells in that their depolarizing phase is larger than the hyperpolarizing phase (Russell, Richardson & Cody, 1986). A parsimonious explanation for the amplitude- and frequency-dependent symmetry of the responses of outer hair cells in the basal turn of the guinea-pig cochlea is that they are determined by their mechanical input, which according to Davis (1958) is the shear displacement of the tectorial membrane.

Unfortunately this explanation is not a simple one because of the probability that the mechanical input to the inner and outer hair cells is determined, at least in part, by the non-linear characteristics of the outer hair cells as a consequence of their proposed mechano-electrical interaction with the tectorial membrane (see Mountain,

1986, for a review). In this respect, a recent model for generating hair-cell receptor potentials, which incorporates voltage-sensitive stiffness in the responses of the stereocilia bundles to angular displacement (McMullen & Mountain, 1985), produces voltage responses to low-frequency tones very similar to those we have observed *in vivo* in outer hair cells. Thus the mechanical stimulus to the hair cells may be controlled by the passive and active non-linear properties of the outer hair cells and the tectorial membrane. This hypothesis has been examined to a limited extent in experiments which show that, apart from their amplitude, the low-frequency wave forms of inner hair-cell receptor potentials remain unchanged by current injection whereas the outer hair-cell receptor potentials are considerably altered (Russell *et al.* 1986). Further investigation is necessary to see if these changes are associated with changes in the mechanical input to the outer hair cells or, indeed, if the non-linear responses of outer hair cells to current injection are the basis for the amplitude- and frequency-dependent properties of their receptor potential wave forms.

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